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(54) **Octapeptide or heptapeptide derivatives, a process for preparing them as well as medicaments containing these compounds and the use of them.**

(57) A subject matter of the invention are octapeptide or heptapeptide amide derivatives of formula

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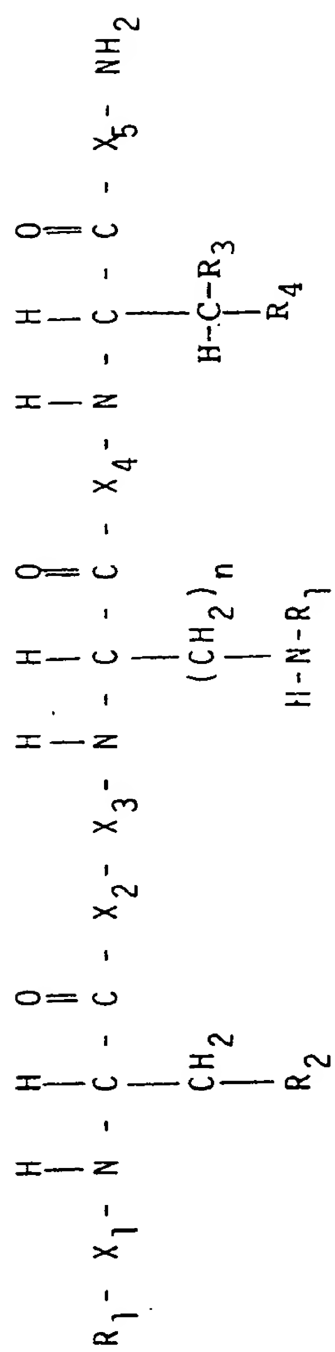
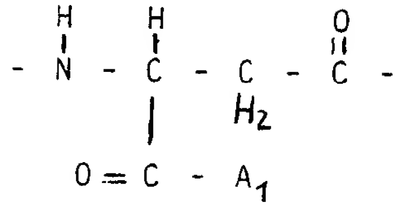


I,

wherein

X_1 stands for an aromatic D-amino acid rest: or a D-phenylglycyl, p-hydroxyphenylglycyl, o-aminobenzoyl, m-aminobenzoyl, D-tetrahydroisoquinolylcarbonyl or sarcosylalanyl; or a



group,
in which latter

A_1 means the rest of an amine
or means an amino group
or means an alkoxy, aryloxy or aralkyloxy group;

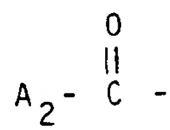
X_2 represents an aromatic amino acid rest or a histidyl group;

X_3 means a D-tryptophyl, o-aminobenzoyl, m-aminobenzoyl, aspartyl, D-aspartyl, β-aspartyl, β-D-aspartyl or aminosuccinyl group; or a group indicated for X_1;

X_4 stands for an amino acid rest bearing an alkyl or aralkyl side chain; or a prolyl or β-alanyl group; or a valence bond;

X_5 means an aromatic amino acid rest or a threonyl group;

R_1 represents hydrogen or a



group,
in which latter

A_2 means hydrogen or an alkyl group;

R_2 means hydrogen, -S- or a -S-acetamidomethyl, phenyl or p-hydroxyphenyl group;

R_3 stands for hydrogen or hydroxyl;

R_4 means a phenyl or p-hydroxyphenyl group; or -S-or a -S-acetamidomethyl group when the meaning of R_2 is the same group; or a methyl group when R_3 is hydroxyl and

n is 1, 2, 3 or 4

and acid addition salts thereof.

The invention is concerned with novel octapeptide or heptapeptide amide derivatives, a process for preparing them as well as medicaments containing these compounds and the use of them.

Somatostatin, a cyclic tetradecapeptide (SRIF), being an inhibitor of secretion of the growth hormone (GH) was originally isolated from the hypothalamus [Brazeau et al.: Science 179, 77 (1973)]. Somatostatin has a very broad spectrum of biological effects, participates in a high number of biological processes and in the majority of cases it plays a role of an inhibitory factor (it inhibits e.g. the release of prolactin, insulin, glucagon, gastrin, secretin and cholecystoquinine) [S. Reichlin: Somatostatin, N. Eng. J. Med. 309, 1495 and 1556 (1983)].

One of the most important effects of somatostatin being a growth-inhibiting factor consists in its capability to influence various forms of pathological cell growth. It is well known from the literature that it exerts an inhibitory action on the growth of cancerous cells [A. V. Schally: Cancer Rs. 48, 6977 (1988); Taylor et al.: Biochem. Biophys. Res. Commun. 153, 81 (1988)]. Likely, somatostatin exerts its antagonizing action on growth factors related to cancerous processes. It has been shown by recent investigations that somatostatin and some somatostatin analogues are capable to activate the tyrosine phosphatase enzyme which antagonizes the effect of tyrosine kinases playing a very important role in the tumorous transformation [A. V. Schally: Cancer Res. 48, 6977 (1988)]. The importance of tyrosine kinases is supported by the fact that the majority of oncogens codes for tyrosine kinase and the major part of the growth factor receptors is tyrosine kinase [Yarden et al.: Ann. Rev. Biochem. 57, 443 (1989)].

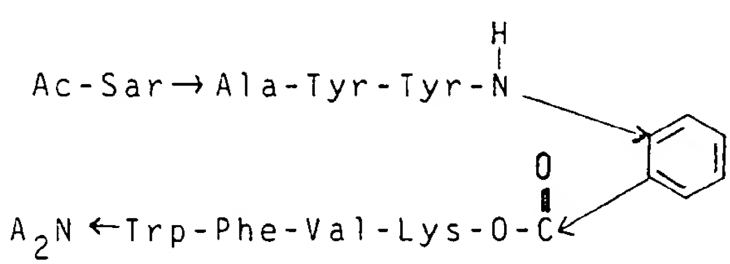
Native somatostatin has a very short duration of effect in vivo since it is rapidly inactivated by endo- and exopeptidases. A high number of novel analogues have been prepared in order to enhance the duration of effect, biological activity and selectivity of this hormone. Most of the active analogues contain a cycle and a peptide chain which is shorter than the original one. The first cyclic hexapeptide showing the whole effects of somatostatin was synthetized by Veber et al. [Nature 292, 55 (1981)]. As a continuation newer and more effective cyclic hexa- and octapeptides have been synthetized which possess the whole spectrum of effects of somatostatin [Veber et al.: Life Sci. 34, 1371 (1984); Murphy et al.: Biochem. Biophys. Res. Commun. 132, 922 (1985); Cai et al.: Proc. Natl. Acad. Sci. USA 83, 1896 (1986)].

The problem underlying to the invention is to create novel somatostatin analogues showing a more advantageous and/or more selective pharmacological action, particularly in treating mammals, including man, suffering from tumour disease, in comparison to that of the known compounds, a process for preparing them as well as medicaments containing these compounds and the use of them.

Surprisingly this has been solved by the invention.

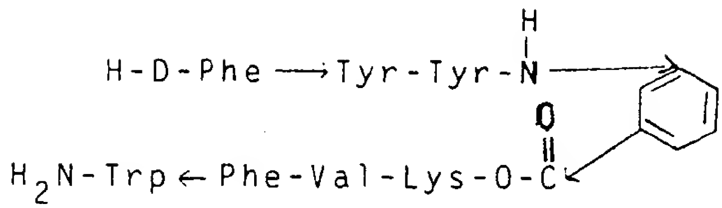
The invention is based on the recognition that the effect of octapeptide and heptapeptide analogues of somatostatin can be strengthened when the amino acid in 1-position is replaced by a substituent of strongly hydrophobic character and having a structure inhibiting the activity of exopeptidases. This substitution can preferably be combined with the replacement of the amino acid in 3-position by various aromatic or heterocyclic amino acids; or with the replacement of the amino acid in 4-position by an aromatic D-amino acid, aromatic aminocarboxylic acid, aminosuccinimide or a β-aspartyl group, optionally substituted on its α-carboxyl group; or with the replacement of the amino acid in 6-position by an amino acid, bearing an alkyl or aralkyl side chain, or substituted derivatives thereof or proline or β-alanine; or with omission of the amino acid in 6-position; or with replacement of the amino acid in 8-position by an aromatic amino acid or a ring-substituted derivative thereof or by threonine.

A further basis of the invention is the recognition that, when an anthranilyl substitution, for example, in compound of formula



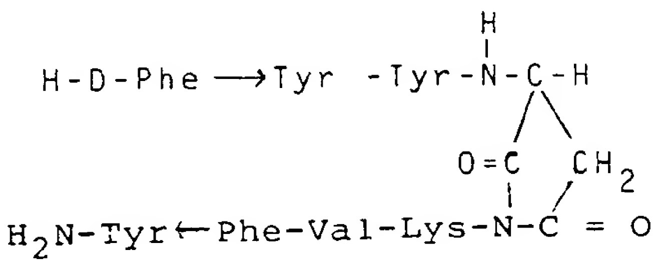
III ,

m-aminobenzoyl substitution, for example, in compound of formula



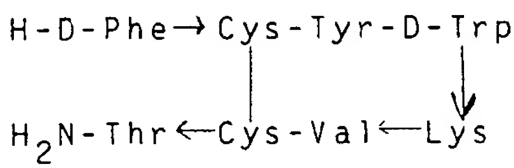
IV ,

or aminosuccinyl substitution, for example, in compound of formula



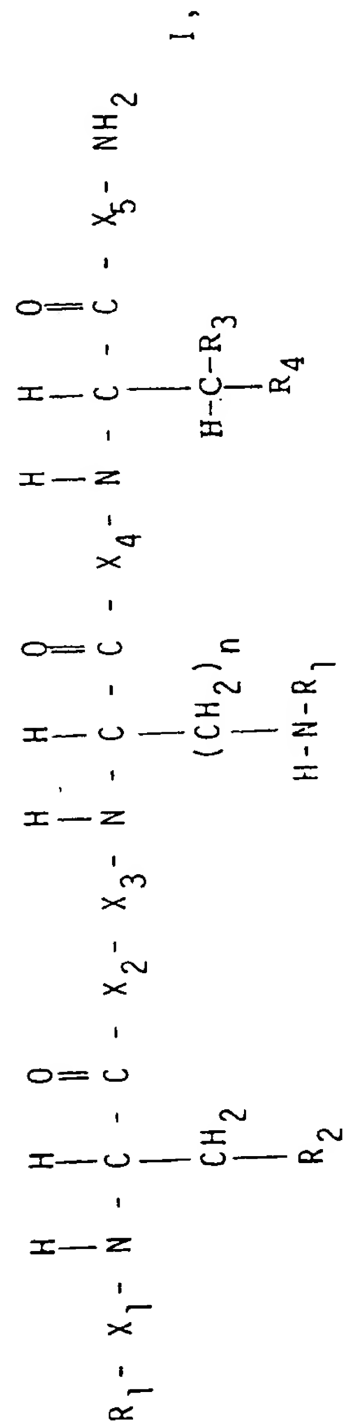
V ,

is used, the compound has not to be cyclized since these replacements inhibit in some cases the free steric rotation of a given peptide (US patent 4,758,552; HU patent 194,280); otherwise the rotation is prevented by the cyclized form, too, for example, in compound of formula



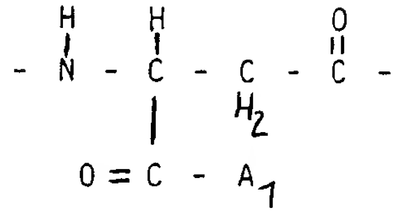
II ,

Thus a subject matter of the invention are octapeptide or heptapeptide amide derivatives of the general formula



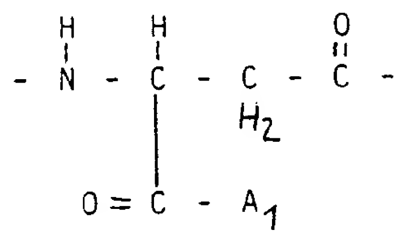
wherein

X_1 stands for an aromatic D-amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a D-phenylglycyl, p-hydroxyphenylglycyl, o-aminobenzoyl, m-aminobenzoyl, D-tetrahydroisoquinolylcarbonyl or sarcosylalanyl; or a



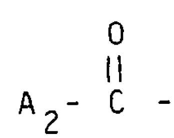
group,
in which latter

- A_1 means the rest of an aromatic, heterocyclic or aliphatic amine having a primary or secondary amino group
or means a primary or secondary aromatic, heterocyclic or aliphatic amino group or means an alkoxy, aryloxy or aralkyloxy group;
- X_2 represents an aromatic amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a histidyl group;
- X_3 means a D-tryptophyl, o-aminobenzoyl, m-aminobenzoyl, aspartyl, D-aspartyl, β-aspartyl, β-D-aspartyl or aminosuccinyl group; or a



group,
in which latter

- A_1 is as defined above;
- X_4 stands for an amino acid rest bearing an alkyl or aralkyl side chain, or a derivative thereof substituted by a hydroxyl or methyl group in β-position; or a prolyl or β-alanyl group; or a valence bond;
- X_5 means an aromatic amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a threonyl group;
- R_1 represents hydrogen or a



group,
in which latter

- A_2 means hydrogen or a C_{1-4} alkyl group, optionally substituted by halogen;
- R_2 means hydrogen, -S- or a -S-acetamidomethyl, phenyl or p-hydroxyphenyl group;
- R_3 stands for hydrogen or hydroxyl;
- R_4 means a phenyl or p-hydroxyphenyl group; or -S-or a -S-acetamidomethyl group when the meaning of R_2 is the same group; or a methyl group when R_3 is hydroxyl, in case of R_2 and R_4 each being -S- one bond each of them are united to a sole bond representing a bridge between R_2 and R_4 and
- n is 1, 2, 3 or 4,

and acid addition salts thereof.

The abbreviations used in the formulae are in agreement with the nomenclature accepted in the peptide

chemistry, which has been published e.g. in J. Biol. Chem. 241, 527 (1966). According to this the abbreviations occurring in the description are as follows:

	X	-X-
Phe	phenylalanine	(phenylalanyl)
Trp	tryptophan	(tryptophyl)
Tyr	tyrosine	(tyrosyl)
Sar	sarcosine	(sarcosyl)
Ala	alanine	(alanyl)
His	histidine	(histidyl)
Val	valine	(valyl)
Thr	threonin	(threonyl)
Pro	proline	(prolyl)
Leu	leucine	(leucyl)
Cys	cysteine	(cysteinyl)

Phg	phenylglycine	(phenylglycyl)
Pop	p-hydroxyphenylglycine	(p-hydroxyphenylglycyl)
Aa	o-aminobenzoic acid	(o-aminobenzoyl)
	anthranilic acid	(anthranilyl)
Mab	m-aminobenzoic acid	(m-aminobenzoyl)
Tic	tetrahydroisoquinoline-carboxylic acid	(tetrahydroisoquinolyl-carbonyl)
Asu	aminosuccinimide	(aminosuccinyl)
Ac	acetyl	
Boc	tertiary butyloxycarbonyl	
Bop	benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate	
tBu	tertiary butyl	
Bzl	benzyl	
DCC	dicyclohexylcarbodiimide	
DCU	dicyclohexylurea	
DIC	diisopropylcarbodiimide	
DIPEA	diisopropylethylamine	
DMF	dimethylformamide	
Et	ethyl	
Fmoc	(9-fluorenylmethyl)-oxycarbonyl	
For	formyl	
HPLC	high performance liquid chromatography	
Me	methyl	
ONP	p-nitrophenyl	
Opcp	pentachlorophenyl	
Opfp	pentafluorophenyl	
Ph	phenyl	
TEA	triethylamine	
TEAA	triethylammonium acetate	
TEAP	triethylammonium phosphate	
TFA	trifluoroacetic acid	
Tfa	trifluoroacetyl	
THF	tetrahydrofuran	
TLC	thin layer chromatography	
Tos	tosyl	
UV	ultraviolet	
Z	benzyloxycarbonyl.	

Preferably the D-amino acid rest which may be represented by X_1 is a D-phenylalanyl , D-naphthylalanyl , preferably D-2-naphthyl -alanyl , or D-tryptophyl rest, above all the first one, and/or the amino acid rest which may be represented by X_2 is a tyrosyl rest and/or the halogen by which the rest of the D-amino acid derivative which may be represented by X_1 and/or the rest of the amino acid derivative which may be represented by X_2 may be ring-substituted is iodine, particularly preferably the rest of the D-amino

acid derivative which may be represented by X_1 being a D-diiodtyrosyl rest and/or the rest of the amino acid derivative which may be represented by X_2 being a diiodtyrosyl rest.

Furthermore it is preferred that the rest of the amine which may be represented by A_1 is an indolinyl or phenylamino rest, the alkoxy group which may be represented by A_1 is such having from 1 to 4, particularly 1 or 2, carbon atom(s), the aryloxy group which may be represented by A_1 is a phenyloxy group or the aralkyloxy group which may be represented by A_1 is such having from 1 to 4, particularly 1 or 2, carbon atom(s) in the alkyl part and having as aryl part a phenyl part.

Moreover it is preferred that the alkyl side chain of the amino acid rest or its derivative which may be represented by X_4 is such having from 1 to 4, particularly 3 or 4, carbon atom(s) or the aralkyl side chain of the amino acid rest or its derivative which may be represented by X_4 is such having from 1 to 4, particularly 1 or 2, carbon atom(s) in the alkyl part and having as aryl part a phenyl part, particularly the amino acid rest which may be represented by X_4 being a leucyl, valyl, isoleucyl, norvalyl, norleucyl, alanyl or seryl rest, above all one of the first two, and/or the aromatic amino acid rest which may be represented by X_5 is a tryptophyl or phenylalanyl rest, above all the first one, or the halogen by which the rest of the derivative of the aromatic amino acid which may be represented by X_5 may be substituted is iodine, particularly preferably the rest of the derivative of the aromatic amino acid which may be represented by X_5 being a tyrosyl or diiodtyrosyl rest, and/or the C_{1-4} alkyl group which may be represented by A_2 is such having 1 or 2 carbon atoms or the halogen by which the C_{1-4} alkyl group which may be represented by A_2 may be substituted is fluorine the number of it being preferably 3, and/or n is 3 or 4, above all the latter one.

Particularly preferred compounds are

ß-aspartyl-(indolinyl)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2, D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys-Thr-NH_2, D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Pop-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2 and Aa-Cys(Acm)-Tyr-D-Trp-Lys-Val-Cys(Acm)-Trp-NH_2.

Suitably the acid addition salts of the octapeptide or heptapeptide derivatives according to the invention are such with pharmaceutically acceptable acids.

A subject matter of the invention is also a process for the preparation of the compounds according to the invention which is characterized in that using the method of the solid-phase peptide synthesis, the protected amino acids are stepwise condensed onto the solid resin carrier through carbodiimide or active ester coupling in the corresponding succession, then the final product is removed from the solid carrier by acidic or alkaline cleavage and the protective groups are removed from the amino acids simultaneously with or before or after cleavage from the resin and, if desired, the octapeptide or heptapeptide amide of the general formula I thus obtained is converted to an acid addition salt by reacting it with an acid and/or, if desired, the free base is liberated from the acid addition salt obtained by reacting it with an alkali and, if desired, the latter is converted to another acid addition salt by reacting it with an acid.

It is suitable to use a benzhydrylamine resin or a chloromethylated polystyrene resin as solid carrier in the process of the invention.

The final product containing protective groups can preferably be splitted off from the resin by using hydrogen fluoride and/or ammonolysis

As already mentioned, the compounds of the invention have superior pharmacological activity, particularly in treating mammals, including man, suffering from tumour disease, and inhibiting tyrosine kinase activity.

Hence by the invention also there are provided for medicaments which are characterized in that they contain as active ingredient(s) 1 or more compound(s) according to the invention, suitably in admixture with 1 or more carrier(s) and/or additive(s) commonly used in the pharmaceutical technique.

The medicaments according to the invention can be in the form of pharmaceutical preparations. These preparations can be prepared by using methods known per se, and thus suitably by mixing 1 or more compound(s) according to the invention with 1 or more carrier(s) and/or additive(s) commonly used in the pharmaceutical technique and transforming the mixture thus obtained to a pharmaceutical preparation.

The compounds according to the invention were found to be active in the dosage range of 0.01 to 500 µg/kg of body-weight (hereinafter:µg/kg) on mice or 0.5 to 2000 mg/kg on man, respectively.

Hence a further subject matter of the invention is the use of the compounds according to the invention for preparing medicaments, particularly for treating mammals, including man, suffering from tumour disease and/or for inhibiting their tyrosine kinase activity.

The pharmacological effects of the compounds according to the invention are supported by the tests described hereinafter.

A) Assay of the growth hormone (GH)

The release or inhibition, respectively, of the release of GH were measured on rat hypophysis by using the superfusion method [Vigh et al.: Peptides 5, 241 (1984)]. As control, the GH amount was considered which was released by the GH releasing hormone (GHRH) given in the same dose as the sample.

The activity of the compound according to the invention was expressed as the percentage of decrease or increase, respectively, in the GH amount released by GHRH (Table I).

B) Assay of the inhibition of cell division

The incorporation of [^{3}H]-thymidine to tumour cells of various origin and measurement of the cell count were carried out according to the method of Kéri et al. [Tumor Biology 9, 315 (1988)]. The biological activity of the compounds according to the invention was expressed as the percentage of inhibition of the labelled thymidine incorporation or increase in the count of untreated cells used as control (Table II).

C) Measurement of the tyrosine kinase activity

The tyrosine kinase activity was also determined according to the method of Kéri et al. [Tumor Biology 9, 315 (1988)]. The activity of the compounds according to the invention was characterized on the basis of their inhibitory effect in comparison to the incorporation of ^{32}P isotope to untreated cells used as control (Table III).

D) Study of effectivity of the compounds on the tumour growth in the metastasis model

The anti-metastatic effect of the compounds was studied on Lewis lung tumour (LLT) cells in muscle-lung and spleen-liver metastasis model as well as on their immunoresistant cell variant (LLT-HH). These experiments were carried out on inbred $C_{57}B1$ mice of both sexes. The LLT was transplanted into the muscle for developing the muscle-lung metastasis model, whereas a suspension of the tumour cells was injected to the spleen to form the spleen-liver metastasis. The compounds to be tested were administered intraperitoneally (i.p.), intravenously (i.v.), orally (p.o.) or subcutaneously (s.c.) in 0.1 to 10 mg/kg doses daily 1 to 3 times in the 5th to 13th days. The therapeutic effect was evaluated on the basis of the number of metastases in such a way that a sample was taken in the spleen-liver model between the 10th and 14th days, in the muscle-lung model between the 17th and 18th days and the macroscopic metastases in the liver and lungs, respectively, were counted under a stereomicroscope. The efficiency of the compounds was expressed in percentage of the count of metastases determined in the control animals (Table IV).

Table I

Change of GH release elicited by GHRH under effect of the analogues of the invention

Compound Designation	Example	Change of GH release
ß-aspartyl-(indolinyl)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	1	-100
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	2	- 93
D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2	3	0
D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2	4	-100
D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys-Thr-NH_2	5	+290
D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	6	+ 26
Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	7	- 20
D-Tic-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	11	- 85

Table I continued

Compound Designation	Example	Change of GH release
D-Phe-Cys-His-D-Trp-Lys-Val-Cys-Thr-NH_2	12	-100
D-Phe-Cys-Tyr-D-Trp-Lys-β-Ala-Cys-Thr-NH_2	14	- 10
D-Phe-Cys-Tyr-D-Trp-Lys-Pro-Cys-Thr-NH_2	15	0
Ac-Sar-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	16	+ 9

Table II

Assay of the inhibition of cell division on various tumour cells

Compound Designation	Example	Cell line+	Dose (μg)	Inhibition (%)
β-aspartyl-(indolinyl)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	1	HT 29 HT 29 MCF 7	1 10 10	36 44 46
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	2	HT 29 MCF 7	20 20	27 45
D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2	3	HT 29 DU 145 PC 3	20 10 10	75 68 45
D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2	4	HT 29 HT 29 MCF 7 DU 145	1 10 10 10	57 50 31 36
D-Phe-Cys-Tyr-β-Asp(indolinyl)-Lys-Leu-Cys-Thr-NH_2	5	SW 620	10	68
D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	6	MCF 7	40	48
Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	7	HT 29 MCF 7	10 20	28 24
D-Tic-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	11	MCF 7	10	21

Table II continued

Compound Designation	Example	Cell line+	Dose (µg)	Inhibition (%)
D-Phe-Cys-His-D-Trp--Lys-Val-Cys-Thr-NH_2	12	MCF 7 MCF 7	10 20	38 44
D-Phe-Cys-Tyr-D-Trp--Lys(Tfa)-Val-Cys-Thr--NH_2	13	MCF 7 MCF 7	10 20	13 19
D-Phe-Cys-Tyr-D-Trp--Lys-ß-Ala-Cys-Thr-NH_2	14	MCF 7 HT 29	20 20	45 13

+ HT 29: tumour cell line of human colon origin
MCF 7: tumour cell line of human breast origin
DU 145: tumour cell line of human prostate
PC 3: tumour cell line of human prostate
SW 620: tumour cell line of human colon origin

Table III

Assay of the tyrosine kinase activity

Compound Designation	Compound Example	Cell line+	Dose (µg)	Inhibition (%)
β-aspartyl-(indolinyl)--Cys-Tyr-D-Trp-Lys-Val--Cys-Thr-NH_2	1	HT 29	10	44
D-Phe-Cys-Tyr-D-Trp--Lys-Val-Cys-Thr-NH_2	2	HT 29 HT 29	1 10	35 22
D-Phe-Cys-Tyr-D-Trp--Lys-Cys-Thr-NH_2	3	SW 620 SW 620	1 10	84 70
D-Phe-Cys-Tyr-D-Trp--Lys-Leu-Cys-Thr-NH_2	4	HT 29 HT 29	10 30	50 90
D-Phe-Cys-Tyr-β-Asp(indolinyl)-Lys-Leu-Cys--Thr-NH_2	5	HT 29 HT 29	10 30	30 45
Ac-Sar-Ala-Tyr-Tyr-Aa--Lys-Val-Phe-Trp-NH_2	7	HT 29 HT 29	10 30	17 23

+ HT 29: tumor cell line of human colon origin
SW 620: tumor cell line of human colon origin

Table IV

Effect on the tumour growth in the metastasis model

Compound Designation	Example	Count of metastases (%) related to controll (100%)
ß-aspartyl-(indolinyl)--Cys-Tyr-D-Trp-Lys-Val--Cys-Thr-NH_2	1	43
D-Phe-Cys-Tyr-D-Trp--Lys-Leu-Cys-Thr-NH_2	4	35
D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys--Thr-NH_2	5	25
D-Phe-Cys-Tyr-D-Trp--Lys(Tfa)-Val-Cys-Thr--NH_2	13	51

The main advantages of the invention are as follows:

a) The compounds according to the invention can preferably be utilized to inhibit tumour growth or the activity of tyrosine kinase enzymes playing an important role in the tumorous transformation.

b) The compounds according to the invention with the new amino acid combinations are useful also for regulating the release of growth hormone (GH), insulin, glucagon and prolactin and/or for inhibiting tumour growth.

c) From the compounds according to the invention bearing an aminosuccinyl, o- and m-aminobenzoyl substitution, those containing no disulfide bridge do not exert any inhibitory effect on the GH release but inhibit the growth of tumour cells.

d) The compounds according to the invention can be used to inhibit pathological processes, such as psoriasis, elicited by the pathological proliferation of skin cells.

e) The o- and m-aminobenzoyl substituents are devoid of any centre of asymmetry, therefore no racemization can occur during their synthesis. Thus, the preparation of the final product becomes easier and cheaper.

f) The preparation costs of o- and m-aminobenzoic acid are much lower than those of the D-amino acids playing a similar role in somatostatin analogues known up to now.

The invention is illustrated in detail by the following Examples.

In the Examples, the compounds are prepared by methods commonly used in the solid-phase peptide synthesis (Stewart et al.: Solid Phase Peptide Synthesis, 2nd Edition. Pierce Chemical Company, Rockford, Illinois, 1984). The individual amino acids are stepwise bound in the form of their Boc or Fmoc derivatives to a benzhydrylamine or chloromethylated polystyrene resin by the symmetric anhydride or active ester coupling or by the aid of DCC, DIC or BOP reagents.

The progress of the reaction is evaluated by the ninhydrin test [E. Kaiser et al.: Anal. Biochem. 34, 595 (1970)]. The acylation is repeated when a free amino group is detected. The time demand of coupling depends on the amino acids and varies between 1 and 16 hours.

Both the removal of protective groups and cleavage of the peptide from the resin are preferably carried out in a single step by using anhydrous liquid hydrogen fluoride [S. Sakakibara et al.: Bull. Chem. Soc. Japan 40, 2164 (1967)].

If desired, the crude product obtained by HF cleavage is cyclized to form the disulphide bridge.

The crude product is purified by using Sephadex chromatography and/or preparative HPLC method. The purity of the final product is examined by TLC, analytical HPLC and amino acid analysis. The TLC R_f values are determined on Kieselgel sheets (DC Alufolien, Merck) by using the solvent mixtures listed hereinafter the ratios being by volume:

1. Ethyl acetate/pyridine/acetic acid/water	30:20:6:11
2. Ethyl acetate/pyridine/acetic acid/water	60:20:6:11
3. Butanol/pyridine/acetic acid/water	60:20:6:11
4. n-Butanol/acetic acid/water	4:1:2
5. n-Butanol/acetic acid/water/ethyl acetate	1:1:1:1
6. n-Butanol/acetic acid/water	4:1:1
7. Isopropanol/1 molar acetic acid	2:1
8. Ethyl acetate/pyridine/acetic acid/water	5:5:1:3

In all Examples the percentages of solvents and eluents are by volume.

Example 1

Preparation of

ß-aspartyl-(indolinyl)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 (Mw: 1076)

0.47 g (0.25 mmol) of benzhydrylamine hydrochloride resin (0.54 milliequivalent/g) is swollen in methylene chloride (CH_2Cl_2) for 90 minutes, then the following cycle is repeated after each acylation carried out with the amino acids. The percentages are by volume.

Step	Reagents, procedure	Time of stirring (min)
1	CH_2Cl_2; 3 washings	1
2	Mixture containing 33 % of TFA, 5 % of anisole and 62 % of CH_2Cl_2; cleavage	1
3	Mixture containing 33 % of TFA, 5 % of anisole and 62 % of CH_2Cl_2; cleavage	1
4	CH_2Cl_2; 3 washings	1
5	Ethanol; 2 washings	1
6	CH_2Cl_2; 3 washings	1
7	Mixture containing 10 % of TEA and 90 % of CH_2Cl_2; 2 washings	2
8	CH_2Cl_2; 3 washings	2
9	Ethanol; 2 washings	1
10	CH_2Cl_2; 3 washings	1
11	3 equivalents of Boc-amino acid dissolved in the mixture of CH_2Cl_2 and DMF as well as 3 equivalents of DCC or DIC dissolved in CH_2Cl_2; coupling	60 minutes to 16 hours
12	CH_2Cl_2; 3 washings	1
13	Ethanol; 2 washings	1

A ninhydrin test is made after each step. When the result is positive, the cycle is repeated from the 6th step.

0.25 mmol of

ß-Asp(indolinyl)-Cys(4-Me-Bzl)-
-Tyr(2-Br-Z)-D-Trp-Lys(Z)-Val-Cys(4-Me-Bzl)-Thr(Bzl)

peptide resin is suspended in 3 ml of anisole containing 10 mixed % of p-cresol, and 30 ml of HF gas are condensed onto the mixture. After stirring at 0 °C for 45 minutes HF is removed, the residue is suspended in 200 ml of ethyl acetate, stirred for 15 minutes, then poured into 250 ml of ethyl acetate. After filtration the precipitate is filtered, washed with ethyl acetate, then the peptide is dissolved from the precipitate by filtration, using 500ml of 95 % by volume gas-free acetic acid. Then 9.0 ml of 0.03 M iodine/methanol solution are dropped to the acetic acid solution until it becomes orange yellow. Thereafter, the solution is stirred for one hour. Zinc powder is cautiously added to the solution until the yellow colour disappears, then the zinc powder is filtered and the solution is evaporated. The solid residue is dissolved in 6 ml of 50 % by volume acetic acid and purified on a Sephadex® G-25 column. The elution is followed by using UV absorption measured at 280 nm and TLC. The fractions containing the aimed product are collected, evaporated, then further purified by gradient elution in a preparative HPLC system (column: Whatman Partisil 10 ODS-3 22x250 mm; eluent A: 0.25 N TEAP, pH 2.24; eluent B: 80 % of methanol with 20 % of A). The pure product is desalinized by gradient elution, using 0.02 M NH_4OAc, pH 5 (eluent A) as well as 70

% of methanol with 30 % of eluent A (eluent B) to obtain 120 mg (43 %) of final product. The physical characteristics of this compound are summarized in Table V, the biological effects already have been shown in Tables I, II, III and IV.

Example 2

Preparation of

D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-
-NH_2 (Mw: 1034)

The aimed peptide is synthetized as described in Example 1, starting from 0.5 g (0.27 mmol) of benzhydrylamine hydrochloride resin (0.54 milliequivalent/g), splitted off from the resin, cyclized and purified by using Sephadex chromatography. Solvents used in the HPLC purification are: A: 0.1 % TEAA, pH 4.2; B: 80 % of methanol with 20 % of A. The product is desalinized by several lyophilizations. In this way the aimed peptide is obtained in a yield of 54 mg (19.4 %). The physical characteristics of this compound are summarized in Table V, the biological effects already have been shown in Tables I, II and III.

Example 3

Preparation of

D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2
(Mw: 947)

Starting from 0.47 g (0.25 mmol) of benzhydrylamine hydrochloride resin (0.54 milliequivalent/g) the peptide is synthetized, cyclized and purified as described in Example 1 to give the final product in a yield of 80.5 mg (34 %). The physical characteristics are summarized in Table V, the biological effects already have been shown in Tables I and II.

Example 4

Preparation of

D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2
(Mw: 1060)

Starting from 0.47 g (0.25 mmol) of benzhydrylamine hydrochloride resin (0.54 milliequivalent/g) the aimed peptide is synthetized, cyclized and purified as described in Example 1 to obtain 106 mg (40 %) of final product. The physical characteristics of this compound are summarized in Table V, the results of biological assays already have been shown in Tables II, III, IV and V.

Example 5

Preparation of

D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys-Thr-NH_2 (Mw: 1076)

Starting from 0.47 g (0.25 mmol) of benzhydrylamine hydrochloride resin the aimed peptide is synthetized, cyclized and purified as described in Example 1 to give 94 mg (35.5 %) of final product. The physical characteristics of this compound are summarized in Table V, the results of biological assays are shown in Tables I, II, III and IV.

Example 6

Preparation of

D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2

(Mw: 1192)

Starting from 0.47 g (0.25 mmol) of benzhydrylamine hydrochloride resin the peptide is synthetized analogously as described in Example 1.

The D-Phe-Tyr(2-Br-Z)-Tyr(2-Br-Z)-Aa-Lys(2-Cl-Z)-Val-Phe-Trp peptide resin obtained after the last step is suspended in 3 ml of anisole, 30 ml of gaseous HF are condensed onto the mixture which is then stirred at 0 °C for 45 minutes. After removing the gaseous HF the residue is suspended in 200 ml of abs. ether, stirred for 15 minutes and after filtration the precipitate is washed with ether. Thereafter, the peptide is dissolved from the precipitate with 50 % by volume acetic acid, the solution is evaporated and the thus-obtained D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2 heptapeptide amide is purified by preparative HPLC. Eluent A: 0.25 N TEAP, pH 2.25; eluent B: mixture of 70 % of acetonitrile and 30 % of eluent A. The separation is carried out on the column mentioned in Example 1 by using a mixture containing 30 % of eluent B and 70 % of eluent A. The pure product is desalinized as described in Example 1 to obtain 89 mg (30 %) of final product. The physical characteristics of this compound are summarized in Table V, the biological effects already have been shown in Tables I and II.

Example 7

Preparation of

Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2 (Mw: 1217)

The aimed peptide is prepared by using the process of Example 6 but an Ac-Sar group is coupled to the peptide resin in the last cycle. The cleavage of the product from the resin and the purification are also carried out as described in Example 6 to obtain 85 mg (28 %) of final product. The physical characteristics of the compound are summarized in Table V whereas its biological effects already have been shown in Tables I, II and III.

Example 8

Preparation of

Pop-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2

(Mw: 1194)

This peptide is synthetized as described in Example 1, except that in the 11th step Bop reagent is used in an amount equal to the amino acid as activating agent for coupling the amino acid in the presence of an excess of DIPEA. After cleavage by HF and purification by chromatography, the final product is obtained as described in Example 6 with a yield of 105 mg (35 %). The physical characteristics of the compound are summarized in Table V.

Example 9

Preparation of

Aa-Cys(Acm)-Tyr-D-Trp-Lys-Val-

-Cys(Acm)-Trp-NH_2 (Mw: 1283)

After swelling 0.47 g (0.25 mmol) of benzhydrylamine hydrochloride resin in methylene chloride for 90 minutes, the following cycle is repeated after each acylation carried out with the Fmoc-derivatives of the suitably protected amino acids the percentages being by volume:

Step	Reagents, procedure	Time of stirring (minute)
1	DMF; 3 washings	2
2	Mixture containing 20 % of piperidine and 80 % of DMF; cleavage	2
3	Mixture containing 20 % of piperidine and 80 % of DMF; cleavage	10
4	DMF; 5 washings	2
5	Symmetric anhydride* prepared from 3 equivalents of Fmoc-amino acid or Opfp ester; coupling	60
6	DMF; 3 washings	2

* = Preparation of the symmetric anhydride:
3 equivalents of Fmoc-amino acid are dissolved in a mixture of CH_2Cl_2 and DMF. After adding 1.5 equivalents of DCC the mixture is stirred at room temperatue for 15 minutes. The DCU precipitate is filtered off, the solution is evaporated and the residue dissolved in Dmf is used for coupling.

A ninhydrin test is made after each step. When a positive result is obtained the cycle is repeated from the 5th step. The

Aa-Cys(Acm)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-

-Cys(Acm)-Trp

peptide resin obtained after the last step is treated with HF, purified and desalinized as described in Example 6 to obtain 147 mg (46 %) of final product. The physical characteristics of the compound are

summarized in Table V.

Examples 10 to 15

The compounds of the following formulae, listed hereinafter, are prepared by using the process described in Examples 1 to 5.

A-Cys-B-D-Trp-C-E-Cys-Thr-NH_2

Example	A	B	C	E
10	β-Asp(NH-Ph)	Tyr	Lys	Val
11	D- Tic	Tyr	Lys	Val
12	D-Phe	His	Lys	Val
13	D-Phe	Tyr	Lys(Tfa)	Val
14	D-Phe	Tyr	Lys	β-Ala
15	D-Phe	Tyr	Lys	Pro

The physical characteristics of the above compounds are summarized in Table V, the results of biological assays already have been shown in Tables I, II and V.

Examples 16 and 17

Compounds of the following formulae are prepared as described in Example 6:

A-B-Tyr-Aa-Lys-Val-Phe-Trp-NH_2

Example	A	B
16	Ac-Sar	Tyr
17	D-Phe	Ala

The physical characteristics of the above compounds are summarized in Table V, the results of biological assays already have been shown in Table I.

Examples 18 and 19

The following compounds are prepared by using the process described in Example 1:

A-Cys-Tyr-Aa-Lys-Val-Cys-Trp-NH_2

In Example 18 "A" stands for a D-Phe group, in Example 19 for a D-2-naphthyl-alanyl group. The physical data of the compounds are summarized in Table V.

Example 20

The following compound is prepared by using the process described in Example 1:

Aa-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2

The physical data of the compounds are summarized in Table V.

Example 21

D-Phe-Cys-Tyr-Asu-Lys-Val-Cys-Thr-NH_2

The compound is prepared by using the process described in Example 1 with the exception that after coupling the Lys residue the neutralization is carried out with 5 % by weight diisopropyl-ethylamine in dichloromethane, instead of using TEA. The side-chain protecting group (fluorenylmethyl ester) of Asp is removed from the protected peptide resin by stirring it for 2x20 minutes in a 1:1 mixture of DMF and piperidine. The formation of Asp-β-pentafluorophenyl ester is carried out by stirring the suspension of the peptide resin for 30 minutes in DMF with an excess of pentafluorophenol and diisopropyl carbodiimide. The peptide resin is filtered and washed with DMF. The aspartimide derivative is formed from the pentafluorophenyl ester derivative by stirring the peptide resin in DMF. The formation of the aspartimide ring is followed by measuring the released pentafluorophenol. The UV absorption of a small amount of the filtered reaction mixture is measured at 278 nm using DMF as control. The cleavage of the remaining protecting groups, the cleavage of the peptide from the resin, the formation of the disulfide bridge and the purification of the crude product are carried out using the process described in Example 1. The physical data of the compound are summarized in Table V.

Table V

Physical characteristics of compounds of the invention

Compound Designation	Example	M.p. (°C)	$[\alpha]_D^{20°}$	R_f^1	R_f^2	R_f^3	R_f^4	R_f^5	R_f^6	R_f^7	R_f^8
ß-aspartyl-(indolinyl)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	1		-53	0.62	0.19	0.61	0.55		0.56	0.81	
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	2		-93	0.63	0.19	0.63	0.55		0.58	0.81	
D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2	3		-98.2	0.55			0.41		0.36	0.73	
D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2	4		-43	0.65	0.22	0.66	0.59		0.61	0.81	
D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys-Thr-NH_2	5		-63	0.61	0.19	0.64	0.51		0.55	0.16	
D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	6	156-160	-19.6					0.83	0.69		0.88
Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2	7	168-172	-34					0.70	0.60	0.70	0.60

Table V continued

Compound Designation	Example	M.p. (°C)	$[\alpha]_D^{20°}$	R_f^1	R_f^2	R_f^3	R_f^4	R_f^5	R_f^6	R_f^7	R_f^8
Pop-Tyr-Tyr-Aa-Lys-Val--Phe-Trp-NH_2	8							0,80	0,60		0,90
Aa-Cys(Acm)-Tyr-D-Trp--Lys-Val-Cys(Acm)-Trp--NH_2	9	138-140	-47		0.27			0.84	0.40		0.81
ß-Aspartyl-(phenyl-amino)-Cys-Tyr-D-Trp--Lys-Val-Cys-Thr-NH_2	10			0.63	0.26		0.72				
D-Tic-Cys-Tyr-D-Trp--Lys-Val-Cys-Thr-NH_2	11		-57.4	0.63	0.22	0.64	0.51		0.55	0.80	
D-Phe-Cys-His-D-Trp--Lys-Val-Cys-Thr-NH_2	12		-116.2	0.43	0.04	0.35	0.36		0.14	0.16	
D-Phe-Cys-Tyr-D-Trp--Lys(Tfa)-Val-Cys-Thr--NH_2	13		-68.6	0.85	0.63	0.76	0.75		0.78	0.93	
D-Phe-Cys-Tyr-D-Trp--Lys-ß-Ala-Cys-Thr-NH_2	14		-30.8	0.60	0.01	0.54	0.48		0.43	0.80	

Table V continued

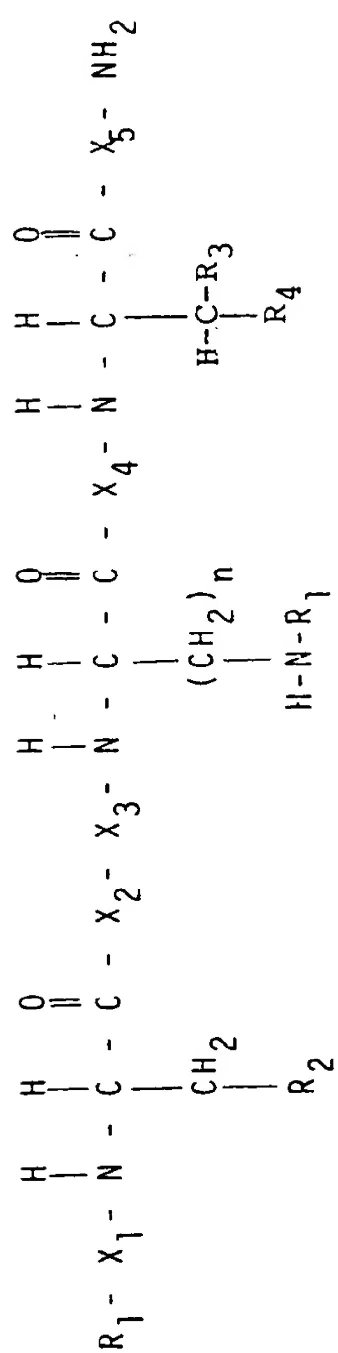
Compound Designation	Example	M.p. (°C)	$[\alpha]_D^{20*}$	R_f^1	R_f^2	R_f^3	R_f^4	R_f^5	R_f^6	R_f^7	R_f^8
D-Phe-Cys-Tyr-D-Trp--Lys-Pro-Cys-Thr-NH_2	15		-63.4	0.57	0.12	0.48	0,41		0.36	0.73	
Ac-Sar-Tyr-Tyr-Aa-Lys--Val-Phe-Trp-NH_2	16			0,57	0.25	0.58			0.57		
D-Phe-Ala-Tyr-Aa-Lys--Val-Phe-Trp-NH_2	17							0,80	0.60		0,90
D-Phe-Cys-Tyr-Aa-Lys--Val-Cys-Trp-NH_2	18							0.61	0.38		
D-2-Naphthyl-Ala-Cys--Tyr-Aa-Lys-Val-Cys--Trp-NH_2	19			0.09				0.74	0.49		
Aa-Cys-Tyr-D-Trp-Lys--Val-Cys-Trp-NH_2	20			0,88					0.79		
D-Phe-Cys-Tyr-Asu-Lys--Val-Cys-Thr-NH_2	21		-31.2		0.13	0.51	0.41				

* c = 0,5 (0,1% by volume acetic acid)

Claims

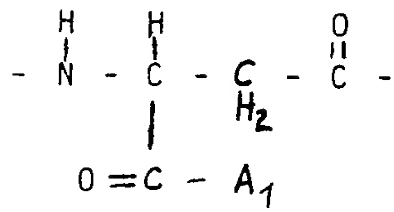
1. Octapeptide or heptapeptide amide derivatives of the general formula

I,



wherein

X_1 stands for an aromatic D-amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a D-phenylglycyl, p-hydroxyphenylglycyl, o-aminobenzoyl, m-aminobenzoyl, D-tetrahydroisoquinolylcarbonyl or sarcosylalanyl; or a



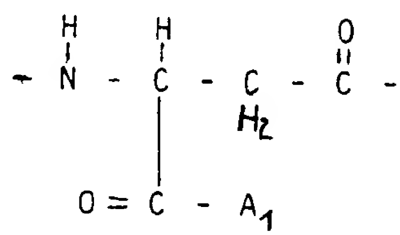
group,

in which latter

A_1 means the rest of an aromatic, heterocyclic or aliphatic amine having a primary or secondary amino group or means a primary or secondary aromatic, heterocyclic or aliphatic amino group or means an alkoxy, aryloxy or aralkyloxy group:

X_2 represents an aromatic amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a histidyl group;

X_3 means a D-tryptophyl, o-aminobenzoyl, m-aminobenzoyl, aspartyl, D-aspartyl, β-aspartyl, β-D-aspartyl or aminosuccinyl group; or a



group,

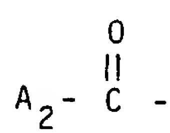
in which latter

A_1 is as defined above;

X_4 stands for an amino acid rest bearing an alkyl or aralkyl side chain, or a derivative thereof substituted by a hydroxyl or methyl group in β-position; or a prolyl or β-alanyl group; or a valence bond:

X_5 means an aromatic amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a threonyl group;

R_1 represents hydrogen or a



group,

in which latter

A_2 means hydrogen or a C_{1-4} alkyl group, optionally substituted by halogen;

R_2 means hydrogen, -S- or a -S-acetamidomethyl, phenyl or p-hydroxyphenyl group;

R_3 stands for hydrogen or hydroxyl;

R_4 means a phenyl or p-hydroxyphenyl group; or -S-or a -S-acetamidomethyl group when the meaning of R_2 is the same group; or a methyl group when R_3 is hydroxyl, in case of R_2 and R_4 each being -S- one bond each of them are united to a sole bond representing a bridge between R_2 and R_4 and

n is 1, 2, 3 or 4,

and acid addition salts thereof.

2. Octapeptide or heptapeptide amide derivatives according to claim 1, characterized in that the D-amino

acid rest which may be represented by X_1 is a D-phenylalanyl, D-naphthylalanyl or D-tryptophyl rest and/or the amino acid rest which may be represented by X_2 is a tyrosyl rest and/or the halogen by which the rest of the D-amino acid derivative which may be represented by X_1 and/or the rest of the amino acid derivative which may be represented by X_2 may be ring-substituted is iodine, particularly preferably the rest of the D-amino acid derivative which may be represented by X_1 being a D-diiodtyrosyl rest and/or the rest of the amino acid derivative which may be represented by X_2 being a diiodtyrosyl rest.

3. Octapeptide or heptapeptide amide derivatives according to claim 1, characterized in that the rest of the amine which may be represented by A_1 is an indolinyl or phenylamino rest, the alkoxy group which may be represented by A_1 is such having from 1 to 4 carbon atom(s), the aryloxy group which may be represented by A_1 is a phenyloxy group or the aralkyloxy group which may be represented by A_1 is such having from 1 to 4 carbon atom(s) in the alkyl part and having as aryl part a phenyl part.

4. Octapeptide or heptapeptide amide derivatives according to claims 1 to 3, characterized in that the alkyl side chain of the amino acid rest or its derivative which may be represented by X_4 is such having from 1 to 4 carbon atom(s) or the aralkyl side chain of the amino acid rest or its derivative which may be represented by X_4 is such having from 1 to 4 carbon atom(s) in the alkyl part and having as aryl part a phenyl part, particularly the amino acid rest which may be represented by X_4 being a leucyl, valyl, isoleucyl, norvalyl, norleucyl, alanyl or seryl rest and/or the aromatic amino acid rest which may be represented by X_5 is a tryptophyl or phenylalanyl rest,
or the halogen by which the rest of the derivative of the aromatic amino acid which may be represented by X_5 may be substituted is iodine, particularly preferably the rest of the derivative of the aromatic amino acid which may be represented by X_5 being a tyrosyl or diiodtyrosyl rest, and/or the C_{1-4} alkyl group which may be represented by A_2 is such having 1 or 2 carbon atoms or the halogen by which the C_{1-4} alkyl group which may be represented by A_2 may be substituted is fluorine and/or n is 3 or 4.

5. A compound as claimed in claims 1 to 4, selected from the group consisting of

ß-aspartyl-(indolinyl)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2, D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys-Thr-NH_2, D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Pop-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2 and Aa-Cys(Acm)-Tyr-D-Trp-Lys-Val-Cys(Acm)-Trp-NH_2.

6. A process for the preparation of the compounds according to claims 1 to 5, characterized in that using the method of the solid-phase peptide synthesis, the protected amino acids are stepwise condensed onto the solid resin carrier through carbodiimide or active ester coupling in the corresponding succession, then the final product is removed from the solid carrier by acidic or alkaline cleavage and the protective groups are removed from the amino acids simultaneously with or before or after cleavage from the resin and, if desired, the octapeptide or heptapeptide amide of the general formula I thus obtained is converted to an acid addition salt by reacting it with an acid and/or, if desired, the free base is liberated from the acid addition salt obtained by reacting it with an alkali and, if desired, the latter is converted to another acid addition salt by reacting it with an acid.

7. A process as claimed in claim 6, characterized in that a benzhydrylamine resin or a chloromethylated polystyrene resin is used as solid resin carrier.

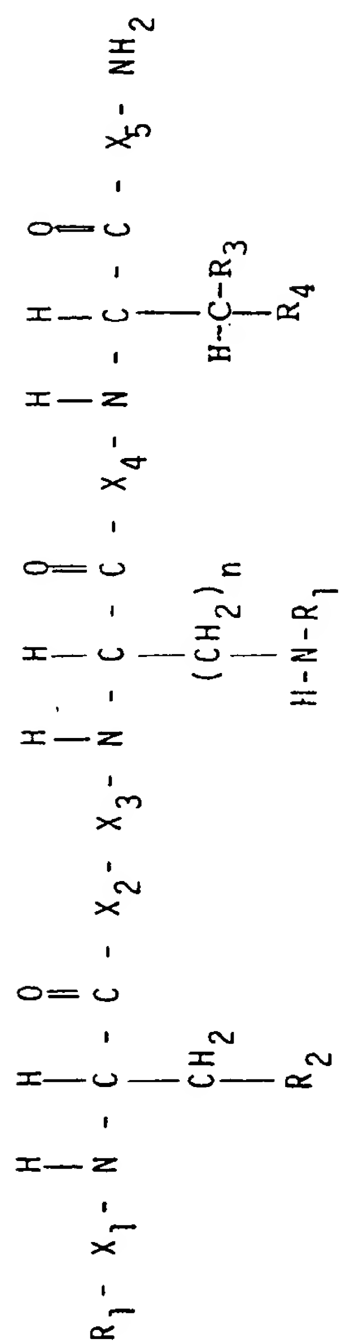
8. A process as claimed in claim 6 or 7, characterized in that the final product containing protective groups is splitted off by hydrogen fluoride and/or ammonolysis.

9. Medicaments characterized in that they contain as active ingredient(s) 1 or more compound(s) as claimed in claims 1 to 8, suitably in admixture with 1 or more carrier(s) and/or additive(s) commonly used in the pharmaceutical technique.

10. The use of the compounds according to claims 1 to 5 for preparing medicaments, particularly for treating mammals, including man, suffering from tumour disease and/or for inhibiting their tyrosine kinase activity.

Claims for the following Contracting States : ES GR

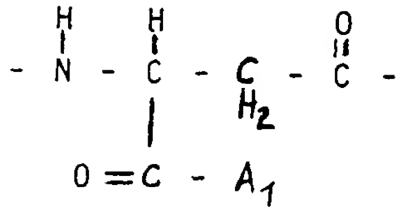
1. A process for the preparation of octapeptide or heptapeptide amide derivatives of the general formula



I,

wherein

X_1 stands for an aromatic D-amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a D-phenylglycyl, p-hydroxyphenylglycyl, o-aminobenzoyl, m-aminobenzoyl, D-tetrahydroisoquinolylcarbonyl or sarcosylalanyl; or a

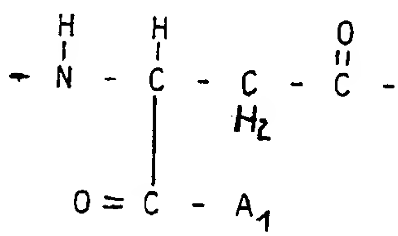


group,
in which latter

A_1 means the rest of an aromatic, heterocyclic or aliphatic amine having a primary or secondary amino group
or means a primary or secondary aromatic, heterocyclic or aliphatic amino group or means an alkoxy, aryloxy or aralkyloxy group;

X_2 represents an aromatic amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl; or a histidyl group;

X_3 means a D-tryptophyl, o-aminobenzoyl, m-aminobenzoyl, aspartyl, D-aspartyl, β-aspartyl, β-D--aspartyl or aminosuccinyl group; or a



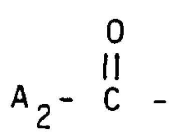
group,
in which latter

A_1 is as defined above;

X_4 stands for an amino acid rest bearing an alkyl or aralkyl side chain, or a derivative thereof substituted by a hydroxyl or methyl group in β-position; or a prolyl or β-alanyl group; or a valence bond;

X_5 means an aromatic amino acid rest or a derivative thereof ring-substituted by halogen and/or hydroxyl: or a threonyl group;

R_1 represents hydrogen or a



group,
in which latter

A_2 means hydrogen or a C_{1-4} alkyl group, optionally substituted by halogen;

R_2 means hydrogen, -S- or a -S-acetamidomethyl, phenyl or p-hydroxyphenyl group;

R_3 stands for hydrogen or hydroxyl;

R_4 means a phenyl or p-hydroxyphenyl group; or -S-or a -S-acetamidomethyl group when the meaning of R_2 is the same group; or a methyl group when R_3 is hydroxyl, in case of R_2 and R_4 each being -S- one bond each of them are united to a sole bond representing a bridge between R_2 and R_4 and

n is 1, 2, 3 or 4,

and acid addition salts thereof,
characterized in that using the method of the solid-phase peptide synthesis, the protected amino acids are stepwise condensed onto the solid resin carrier through carbodiimide or active ester coupling in the

corresponding succession, then the final product is removed from the solid carrier by acidic or alkaline cleavage and the protective groups are removed from the amino acids simultaneously with or before or after cleavage from the resin and, if desired, the octapeptide or heptapeptide amide of the general formula I thus obtained is converted to an acid addition salt by reacting it with an acid and/or, if desired, the free base is liberated from the acid addition salt obtained by reacting it with an alkali and, if desired, the latter is converted to another acid addition salt by reacting it with an acid.

2. A process as claimed in claim 1, characterized in that a benzhydrylamine resin or a chloromethylated polystyrene resin is used as solid resin carrier.

3. A process as claimed in claim 1 or 2, characterized in that the final product containing protective groups is splitted off by hydrogen fluoride and/or ammonolysis.

4. A process as claimed in claims 1 to 3, characterized in that octapeptide or heptapeptide amide derivatives are prepared in which the D-amino acid rest which may be represented by X_1 is a D-phenylalanyl, D-naphthylalanyl or D-tryptophyl rest and/or the amino acid rest which may be represented by X_2 is a tyrosyl rest and/or the halogen by which the rest of the D-amino acid derivative which may be represented by X_1 and/or the rest of the amino acid derivative which may be represented by X_2 may be ring-substituted is iodine, particularly preferably the rest of the D-amino acid derivative which may be represented by X_1 being a D-diiodtyrosyl rest and/or the rest of the amino acid derivative which may be represented by X_2 being a diiodtyrosyl rest.

5. A process as claimed in claims 1 to 3, characterized in that octapeptide or heptapeptide amide derivatives are prepared in which the rest of the amine which may be represented by A_1 is an indolinyl or phenylamino rest, the alkoxy group which may be represented by A_1 is such having from 1 to 4 carbon atom(s), the aryloxy group which may be represented by A_1 is a phenyloxy group or the aralkyloxy group which may be represented by A_1 is such having from 1 to 4 carbon atom(s) in the alkyl part and having as aryl part a phenyl part.

6. A process as claimed in claims 1 to 3, characterized in that octapeptide or heptapeptide amide derivatives are prepared in which the alkyl side chain of the amino acid rest or its derivative which may be represented by X_4 is such having from 1 to 4 carbon atom(s) or the aralkyl side chain of the amino acid rest or its derivative which may be represented by X_4 is such having from 1 to 4 carbon atom(s) in the alkyl part and having as aryl part a phenyl part, particularly the amino acid rest which may be represented by X_4 being a leucyl, valyl, isoleucyl, norvalyl, norleucyl, alanyl or seryl rest and/or
the aromatic amino acid rest which may be represented by X_5 is a tryptophyl or phenylalanyl rest,
or the halogen by which the rest of the derivative of the aromatic amino acid which may be represented by X_5 may be substituted is iodine, particularly preferably the rest of the derivative of the aromatic amino acid which may be represented by X_5 being a tyrosyl or diiodtyrosyl rest,
and/or the C_{1-4} alkyl group which may be represented by A_2 is such having 1 or 2 carbon atoms or the halogen by which the C_{1-4} alkyl group which may be represented by A_2 may be substituted is fluorine and/or n is 3 or 4.

7. A process as claimed in claims 1 to 3, characterized in that octapeptide or heptapeptide amide derivatives are prepared which are selected from the group consisting of

ß-aspartyl-(indolinyl)-Cys--Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D--Trp-Lys-Val-Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys--Cys-Thr-NH_2, D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr--NH_2, D-Phe-Cys-Tyr-ß-Asp(indolinyl)-Lys-Leu-Cys--Thr-NH_2, D-Phe-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Ac-Sar-Ala-Tyr-Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Pop-Tyr--Tyr-Aa-Lys-Val-Phe-Trp-NH_2, Aa-Cys(Acm)-Tyr-D--Trp-Lys-Val-Cys(Acm)-Trp-NH_2.

8. A process for preparing medicaments characterized by mixing as active ingredient(s) 1 or more compound(s) prepared by the process according to claims 1 to 7, with 1 or more carrier(s) and/or additive(s) commonly used in the pharmaceutical technique in a manner knwon per se.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number

EP 92 10 1196

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 203 031 (TULANE) * Whole document, esp. claim 8 * ---	1-10	C 07 K 7/02 C 07 K 7/06 A 61 K 37/02
A	EP-A-0 215 171 (TULANE) * Whole document * ---	1-10	
A	EP-A-0 277 419 (TULANE) * Whole document * ---	1-10	
A	EP-A-0 298 732 (TULANE) * Whole document * ---	1-10	
A	EP-A-0 389 180 (BIOMEASURE) * Whole document * -----	1-10	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 K
A 61 K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely : 5

Claims searched incompletely : 1-4,6-10

Claims not searched :

Reason for the limitation of the search:

The structural formula of claim 1 has no single invariable element. Therefore the search has been restricted to claims 1-10 in so far as defined by claim 5.

Place of search	Date of completion of the search	Examiner
THE HAGUE	02-05-1992	MASTURZO P.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document